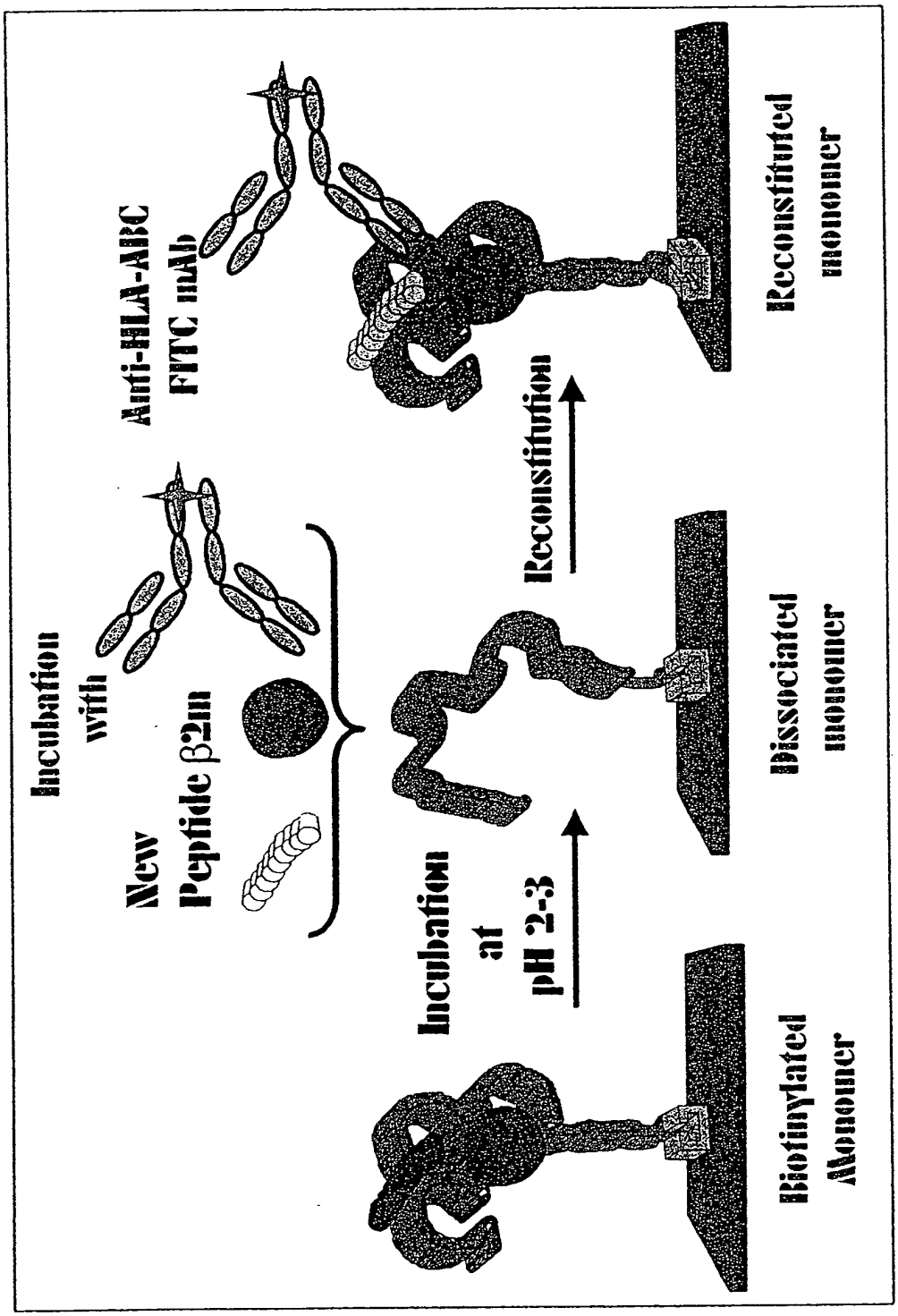

Incubation
with
New
Peptide β2m
Anti-HLA-ABC
FITC mAb
Incubation
at
pH 2-3
Reconstitution
Biotinylated
Monomer
Dissociated
monomer
Reconstituted
monomer

FIG. 1

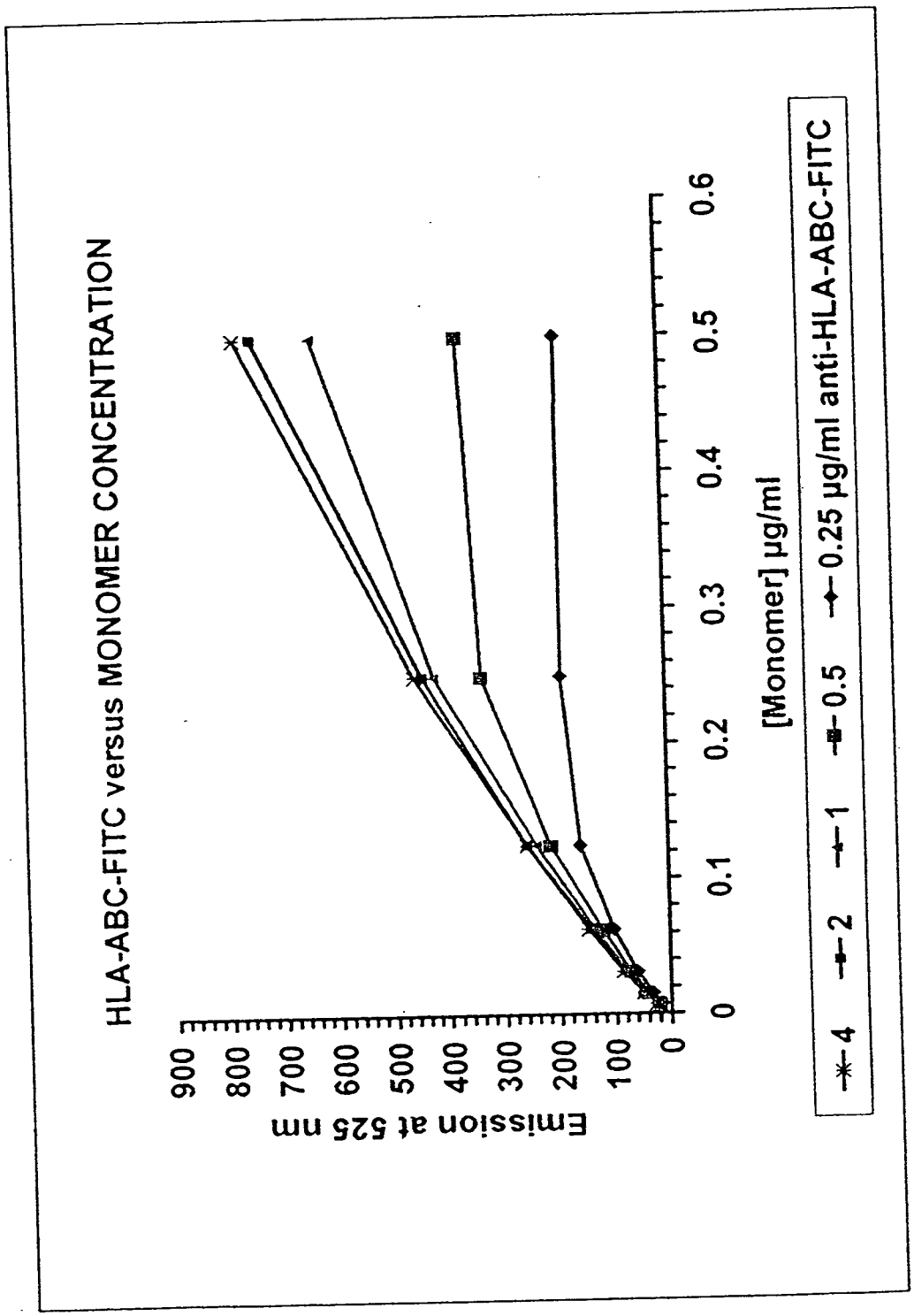

HLA-ABC-FITC versus MONOMER CONCENTRATION
Emission at 525 nm
900
800
700
600
500
400
300
200
100
0
0
0.1
0.2
0.3
0.4
0.5
0.6
[Monomer] µg/ml
4
2
1
0.5
0.25 µg/ml anti-HLA-ABC-FITC

FIG. 2

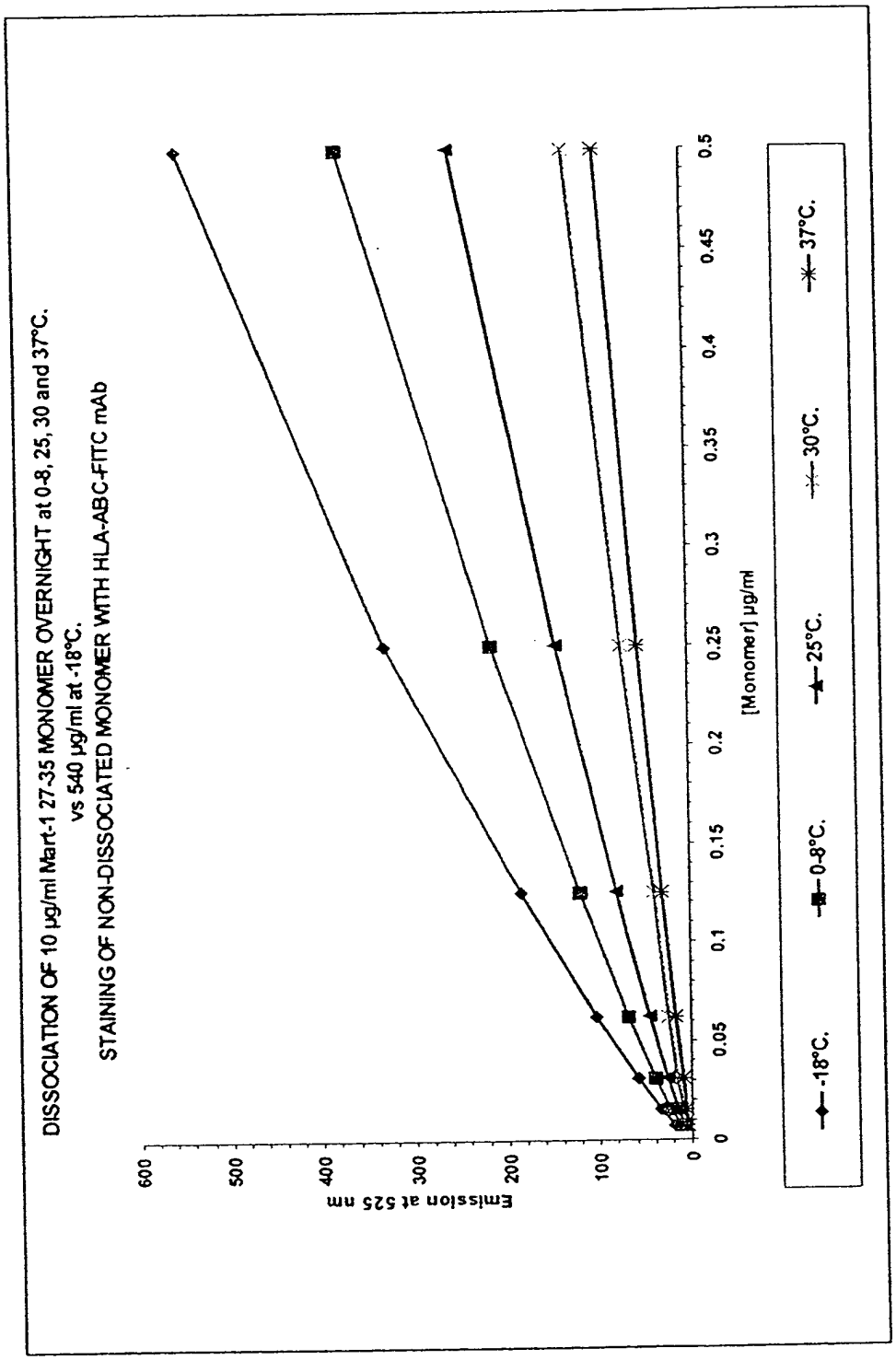

DISSOCIATION OF 10 µg/ml Mart-1 27-35 MONOMER OVERNIGHT at 0-8, 25, 30 and 37°C.
vs 540 µg/ml at -18°C.
STAINING OF NON-DISSOCIATED MONOMER WITH HLA-ABC-FITC mAb
Emission at 525 nm
600
500
400
300
200
100
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
[Monomer] µg/ml
-18°C.
0-8°C.
25°C.
30°C.
37°C.

FIG. 3

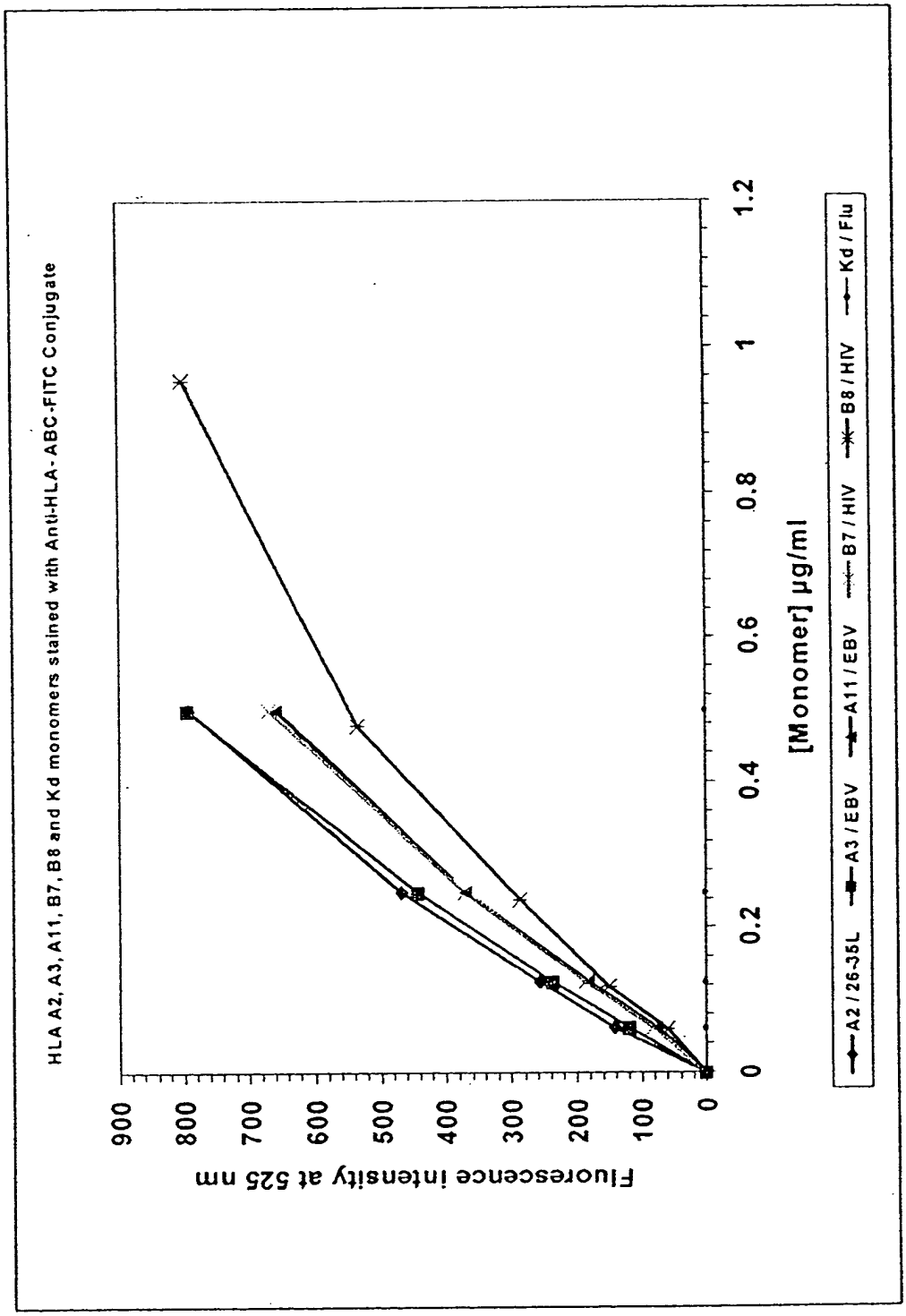

HLA A2, A3, A11, B7, B8 and Kd monomers stained with Anti-HLA-ABC-FITC Conjugate
Fluorescence intensity at 525 nm
900
800
700
600
500
400
300
200
100
0
0
0.2
0.4
0.6
0.8
1
1.2
[Monomer] µg/ml
A2 / 26-35L
A3 / EBV
A11 / EBV
B7 / HIV
B8 / HIV
Kd / Flu

FIG. 4

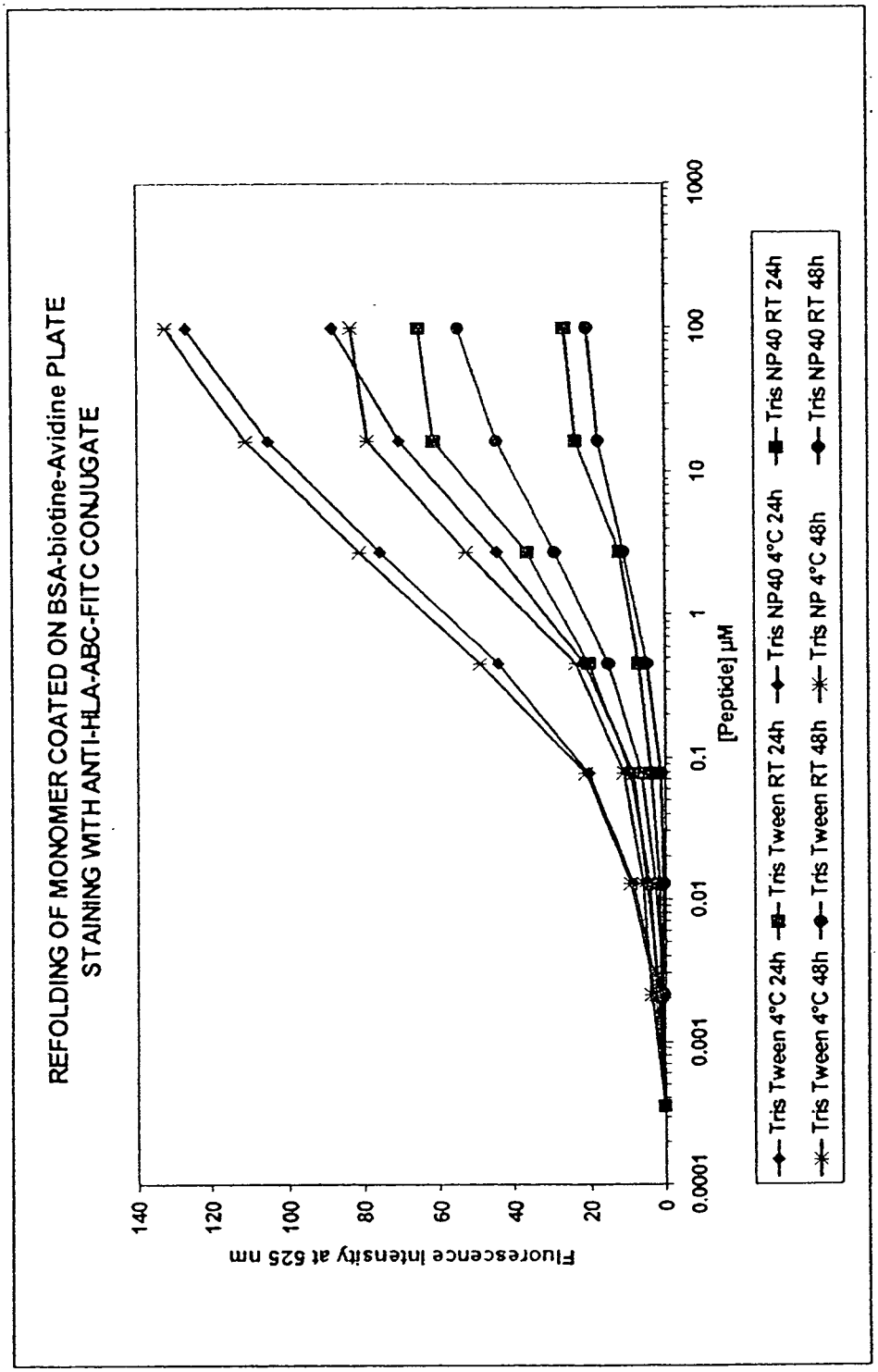

REFOLDING OF MONOMER COATED ON BSA-biotine-Avidine PLATE
STAINING WITH ANTI-HLA-ABC-FITC CONJUGATE
Fluorescence Intensity at 625 nm
0
20
40
60
80
100
120
140
[Peptide] µM
0.0001
0.001
0.01
0.1
1
10
100
1000
Tris Tween 4°C 24h
Tris Tween RT 24h
Tris NP40 4°C 24h
Tris NP40 RT 24h
Tris Tween 4°C 48h
Tris Tween RT 48h
Tris NP 4°C 48h
Tris NP40 RT 48h

FIG. 5

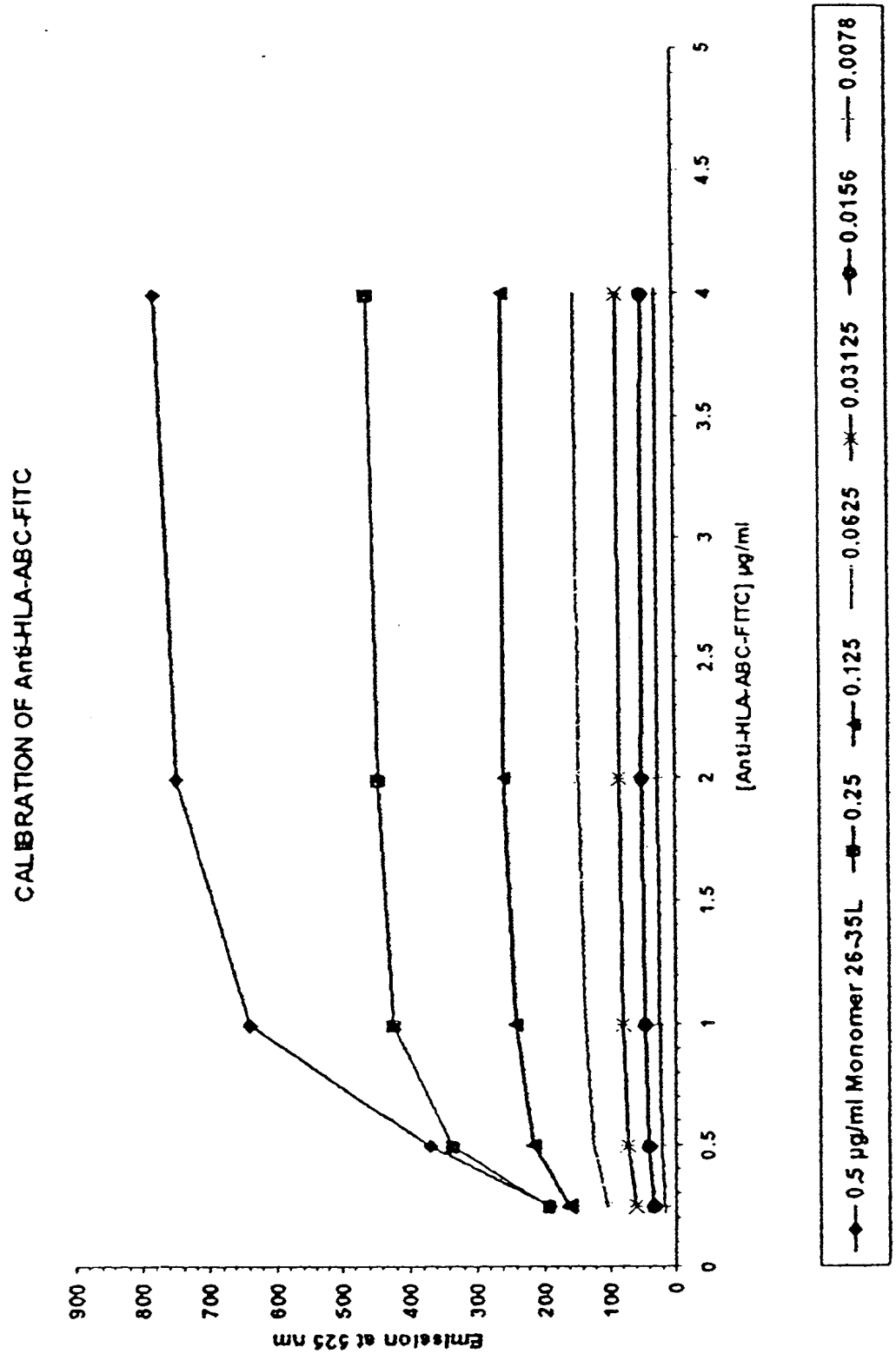

CALIBRATION OF Anti-HLA-ABC-FITC
Emission at 525 nm
0
100
200
300
400
500
600
700
800
900
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
[Anti-HLA-ABC-FITC] µg/ml
0.5 µg/ml Monomer 26-35L
0.25
0.125
0.0625
0.03125
0.0156
0.0078

FIG. 6

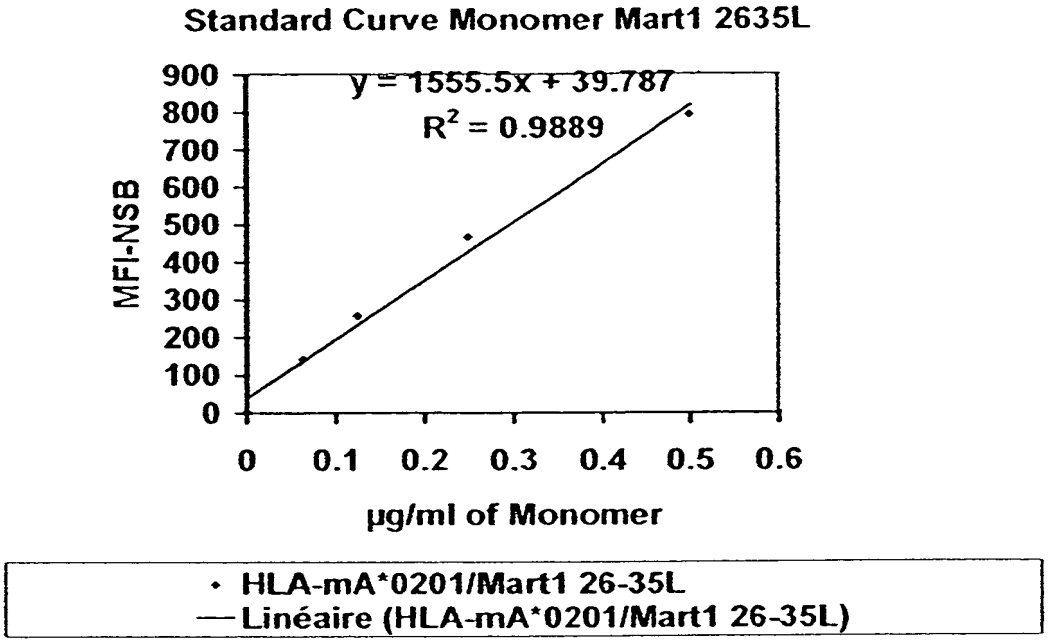

Standard Curve Monomer Mart1 2635L
y = 1555.5x + 39.787
R2 = 0.9889
MFI-NSB
900
800
700
600
500
400
300
200
100
0
0
0.1
0.2
0.3
0.4
0.5
0.6
µg/ml of Monomer
HLA-mA*0201/Mart1 26-35L
Linéaire (HLA-mA*0201/Mart1 26-35L)

FIG. 7A

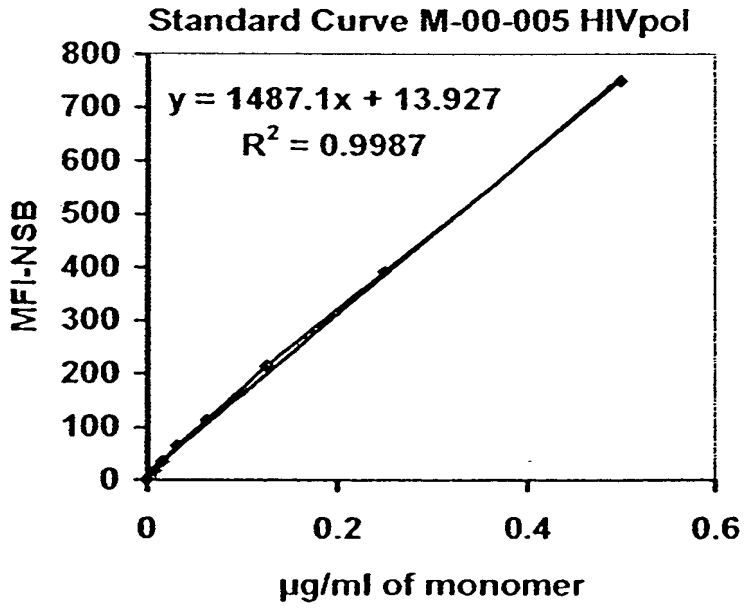

Standard Curve M-00-005 HIVpol
y = 1487.1x + 13.927
R2 = 0.9987
MFI-NSB
800
700
600
500
400
300
200
100
0
0
0.2
0.4
0.6
µg/ml of monomer

FIG. 7B

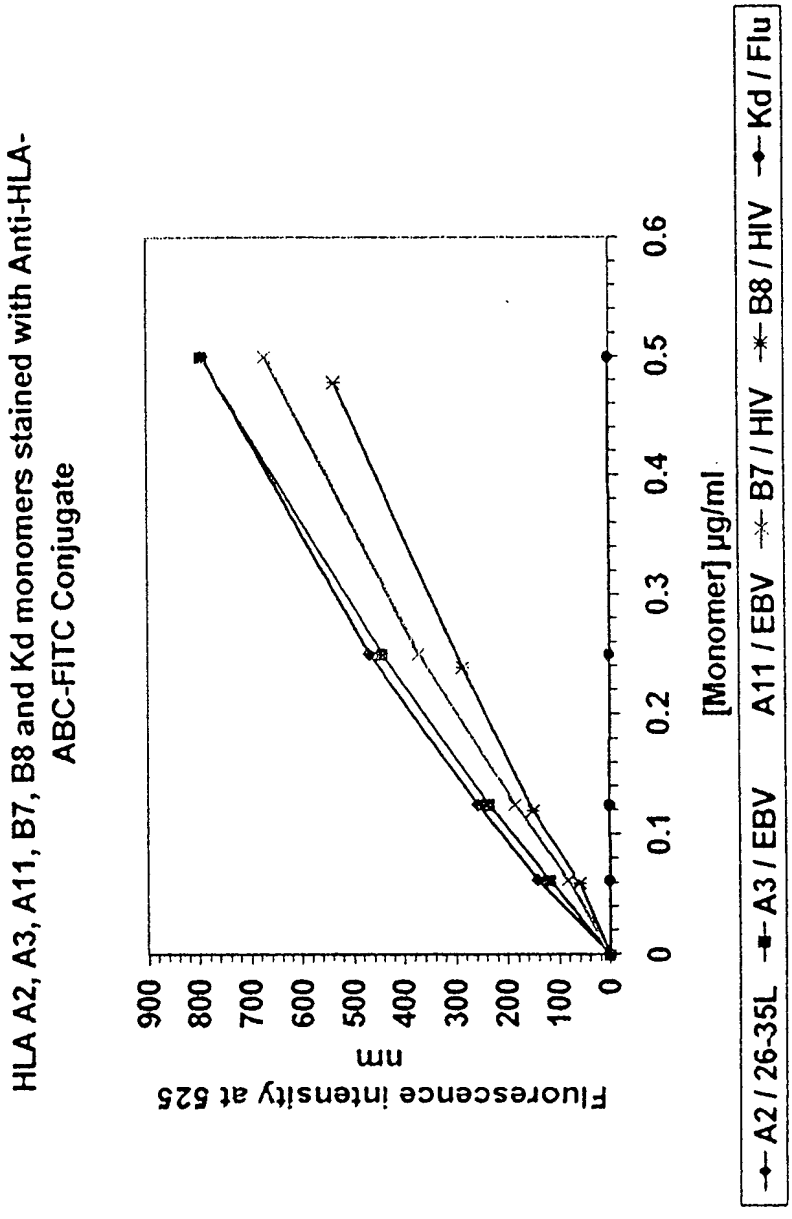

HLA A2, A3, A11, B7, B8 and Kd monomers stained with Anti-HLA-ABC-FITC Conjugate
Fluorescence intensity at 525 nm
900
800
700
600
500
400
300
200
100
0
0
0.1
0.2
0.3
0.4
0.5
0.6
[Monomer] µg/ml
A2 / 26-35L
A3 / EBV
A11 / EBV
B7 / HIV
B8 / HIV
Kd / Flu

FIG. 8

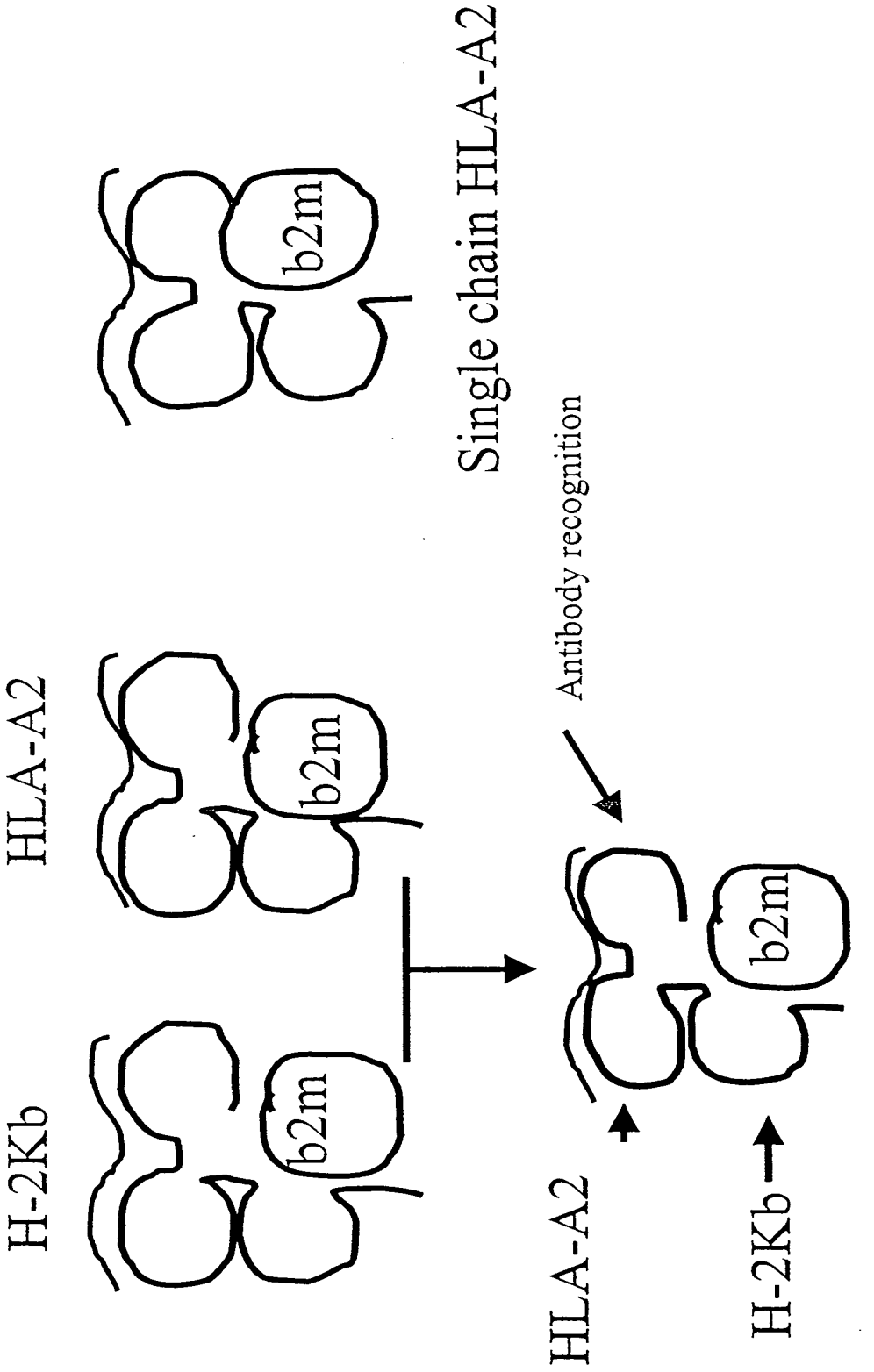

H-2Kb
b2m
HLA-A2
b2m
Single chain HLA-A2
b2m
Antibody recognition
HLA-A2
H-2Kb
b2m

FIG. 9

FIG. 10A

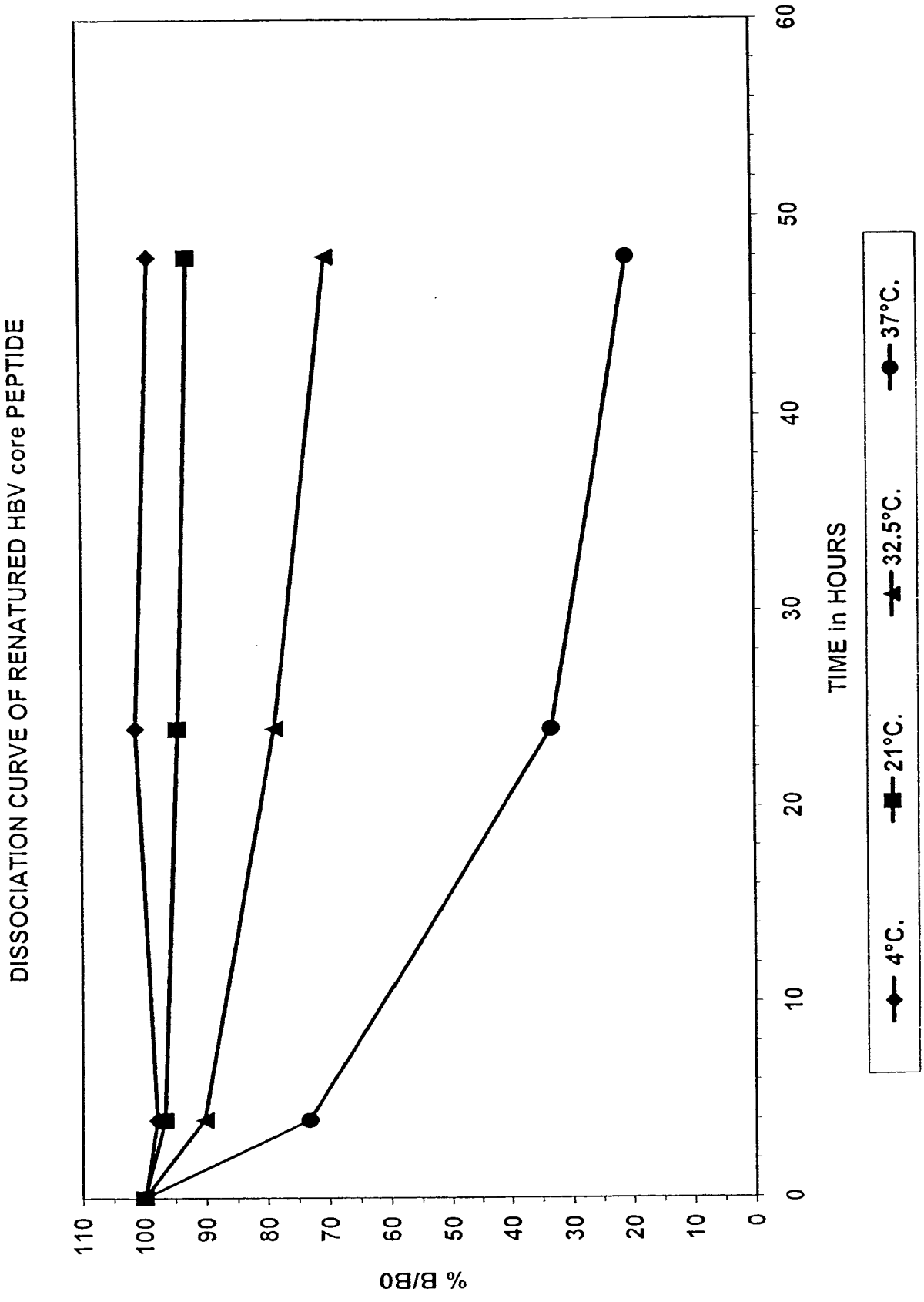

DISSOCIATION CURVE OF RENATURED HBV core PEPTIDE
% B/B0
110
100
90
80
70
60
50
40
30
20
10
0
TIME in HOURS
0
10
20
30
40
50
60
4°C.
21°C.
32.5°C.
37°C.

FIG. 10B

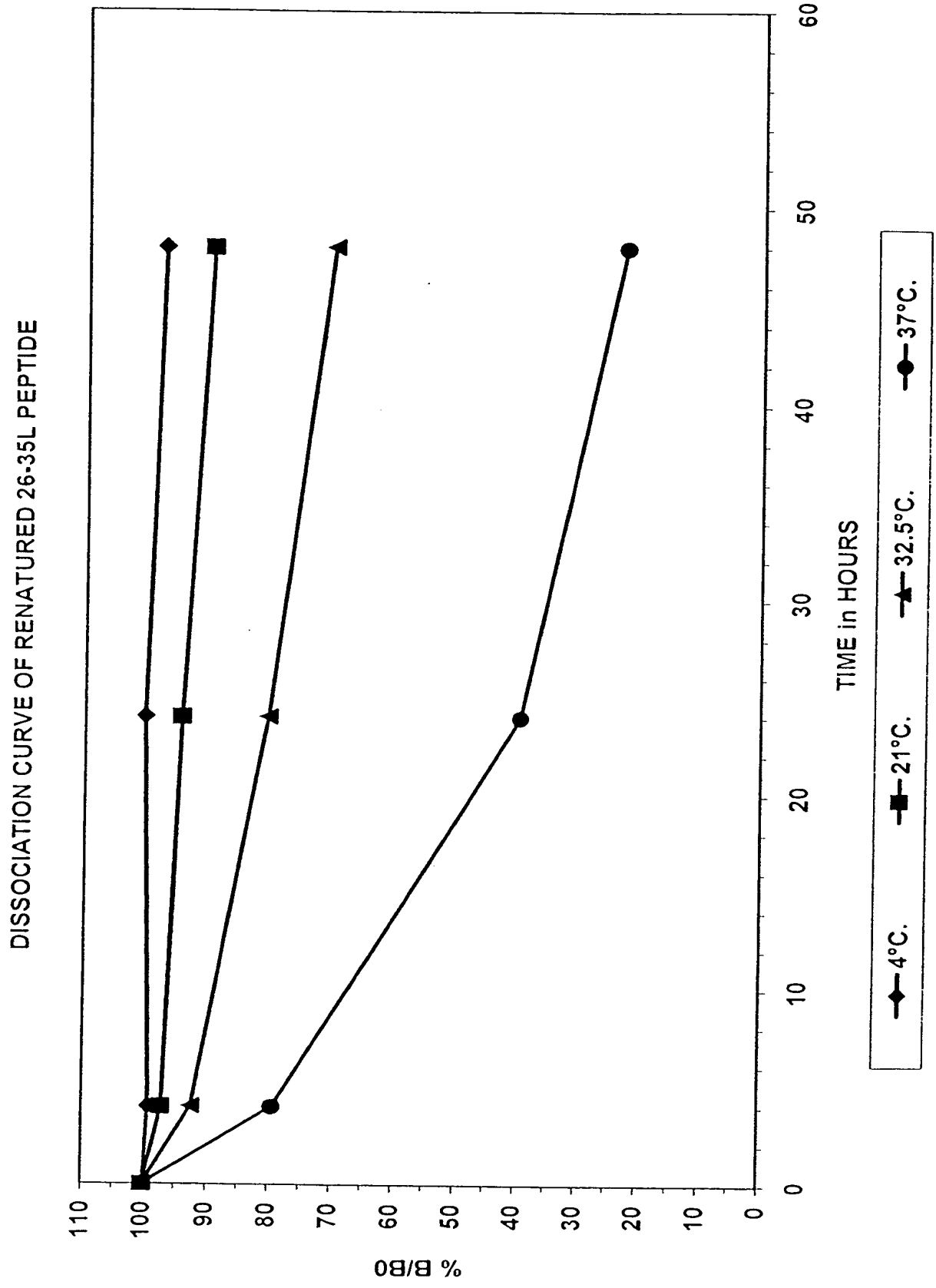

DISSOCIATION CURVE OF RENATURED 26-35L PEPTIDE
% B/Bo
110
100
90
80
70
60
50
40
30
20
10
0
TIME in HOURS
0
10
20
30
40
50
60
4°C.
21°C.
32.5°C.
37°C.

FIG. 10C

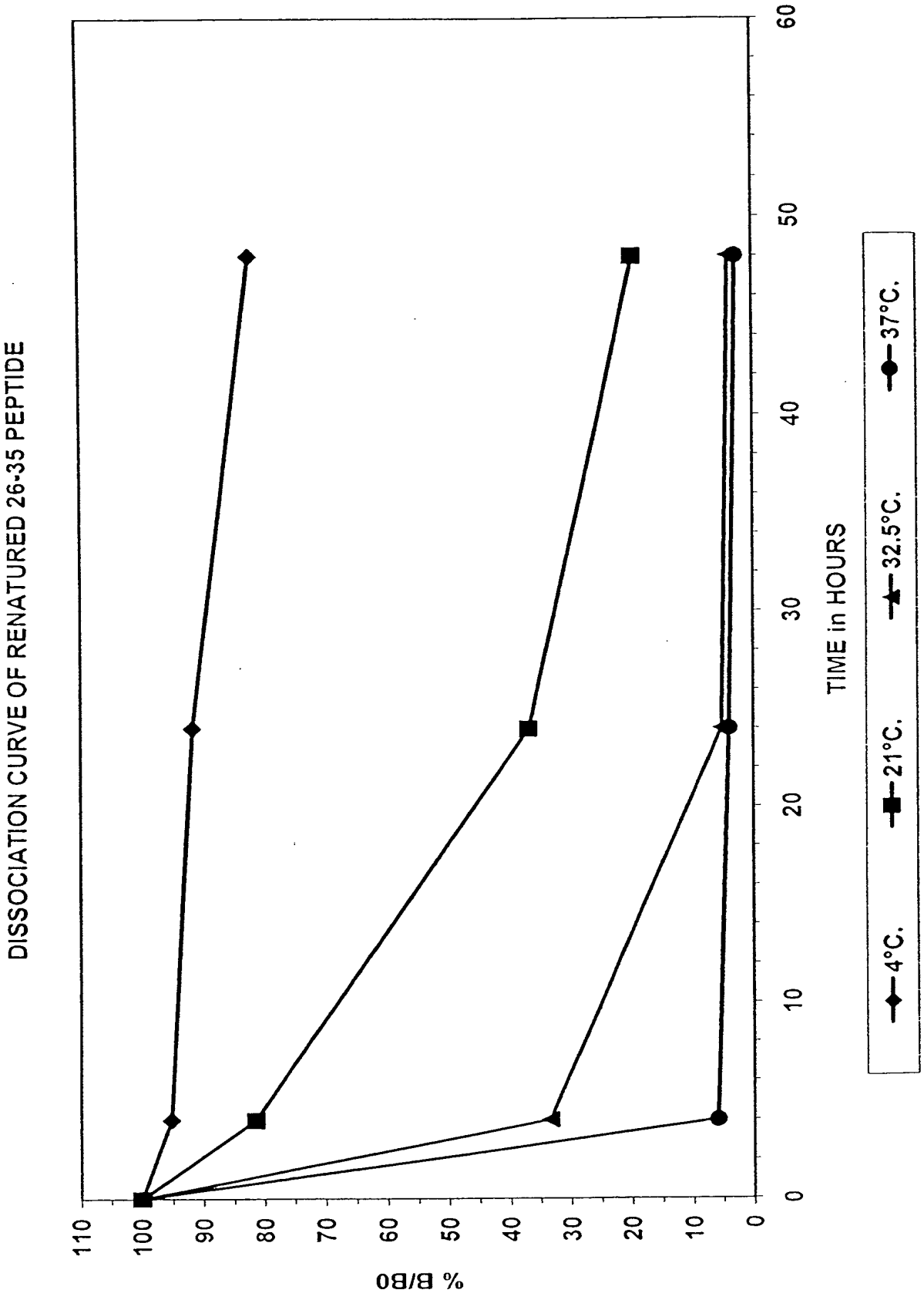

DISSOCIATION CURVE OF RENATURED 26-35 PEPTIDE
% B/B0
110
100
90
80
70
60
50
40
30
20
10
0
TIME in HOURS
0
10
20
30
40
50
60
4°C.
21°C.
32.5°C.
37°C.

FIG. 10D

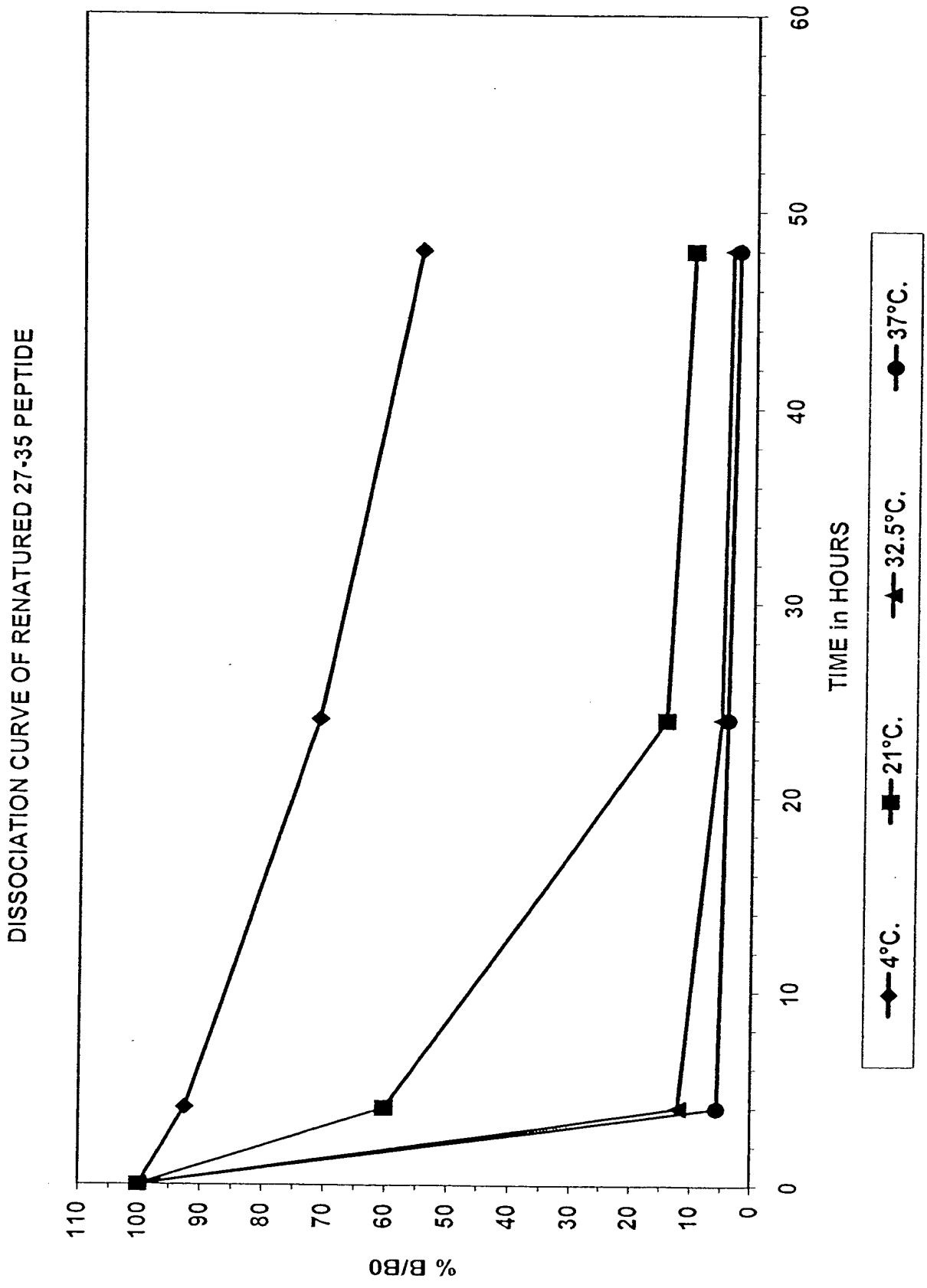

DISSOCIATION CURVE OF RENATURED 27-35 PEPTIDE
% B/B0
110
100
90
80
70
60
50
40
30
20
10
0
TIME in HOURS
0
10
20
30
40
50
60
4°C.
21°C.
32.5°C.
37°C.

FIG. 10E

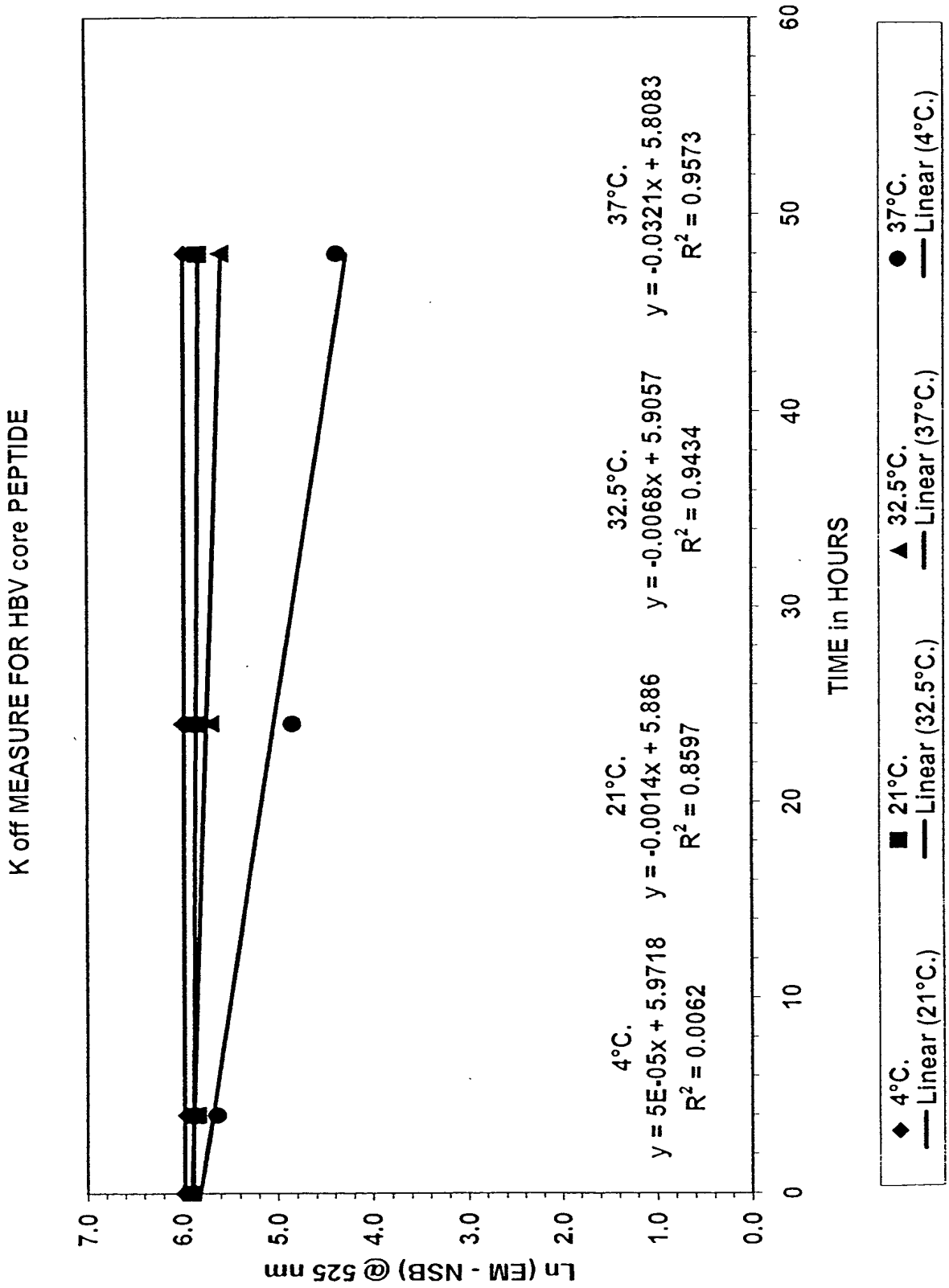

K off MEASURE FOR HBV core PEPTIDE
Ln (EM - NSB) @ 525 nm
TIME in HOURS
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
10
20
30
40
50
60
4°C.
y = 5E-05x + 5.9718
R² = 0.0062
21°C.
y = -0.0014x + 5.886
R² = 0.8597
32.5°C.
y = -0.0068x + 5.9057
R² = 0.9434
37°C.
y = -0.0321x + 5.8083
R² = 0.9573
◆ 4°C.
Linear (21°C.)
■ 21°C.
Linear (32.5°C.)
▲ 32.5°C.
Linear (37°C.)
● 37°C.
Linear (4°C.)

FIG. 10F

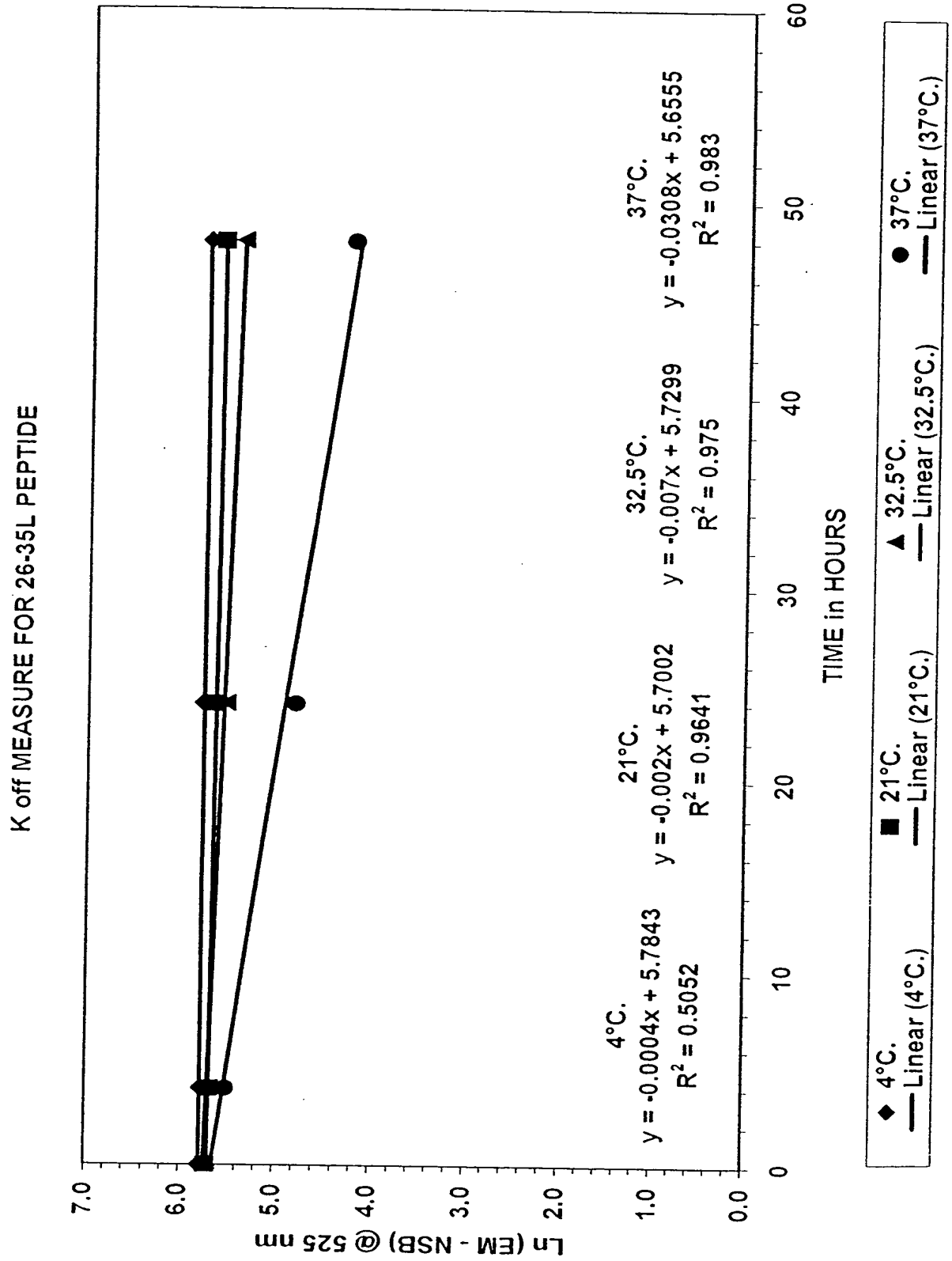

K off MEASURE FOR 26-35L PEPTIDE
Ln (EM - NSB) @ 525 nm
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
10
20
30
40
50
60
TIME in HOURS
4°C.
y = -0.0004x + 5.7843
R² = 0.5052
21°C.
y = -0.002x + 5.7002
R² = 0.9641
32.5°C.
y = -0.007x + 5.7299
R² = 0.975
37°C.
y = -0.0308x + 5.6555
R² = 0.983
◆ 4°C.
Linear (4°C.)
■ 21°C.
Linear (21°C.)
▲ 32.5°C.
Linear (32.5°C.)
● 37°C.
Linear (37°C.)

FIG. 10G

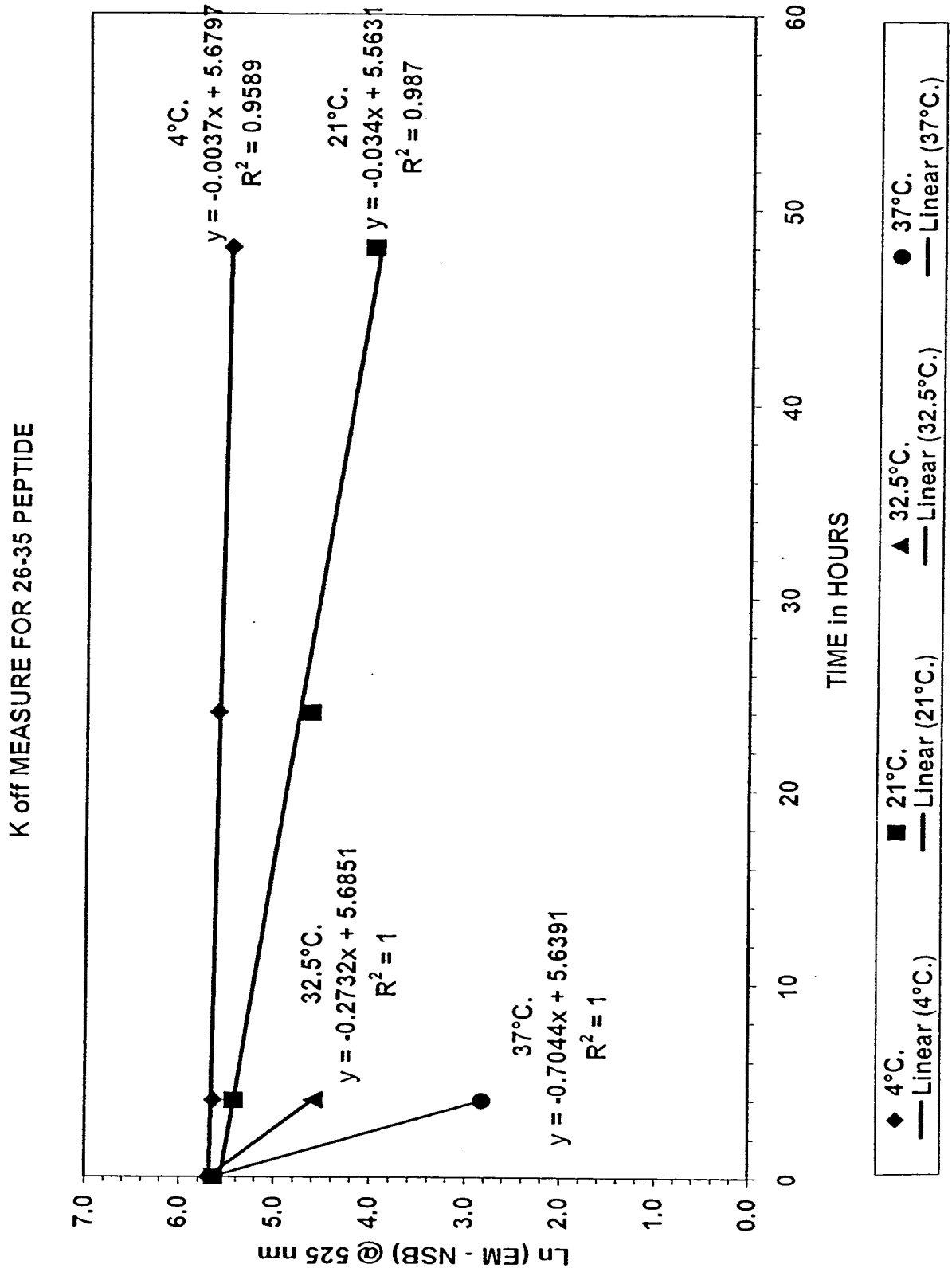

K off MEASURE FOR 26-35 PEPTIDE
Ln (EM - NSB) @ 525 nm
TIME in HOURS
4°C.
y = -0.0037x + 5.6797
R² = 0.9589
21°C.
y = -0.034x + 5.5631
R² = 0.987
32.5°C.
y = -0.2732x + 5.6851
R² = 1
37°C.
y = -0.7044x + 5.6391
R² = 1
4°C.
Linear (4°C.)
21°C.
Linear (21°C.)
32.5°C.
Linear (32.5°C.)
37°C.
Linear (37°C.)

FIG. 10H

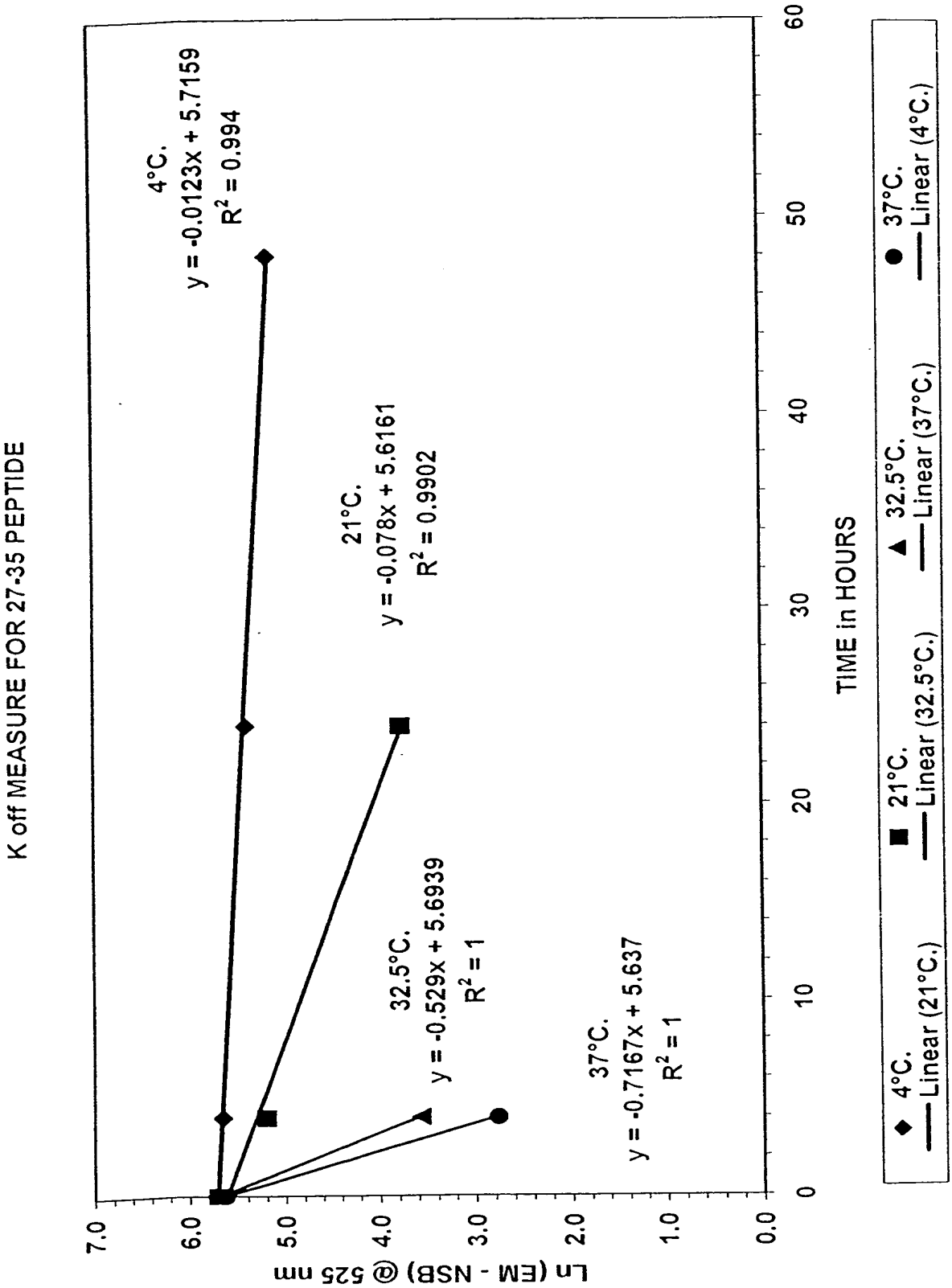
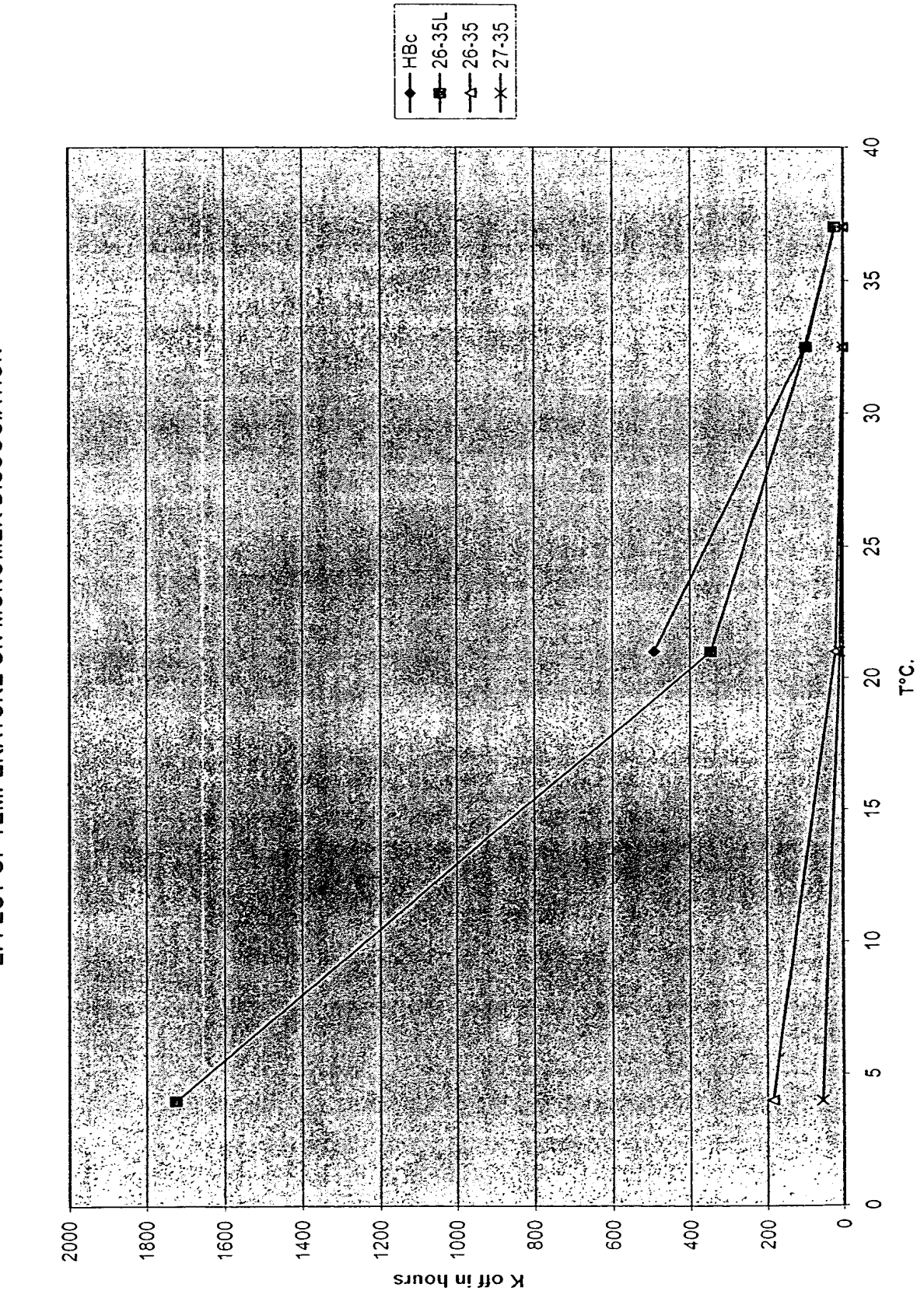

K off MEASURE FOR 27-35 PEPTIDE
Ln (EM - NSB) @ 525 nm
TIME in HOURS
4°C.
y = -0.0123x + 5.7159
R² = 0.994
21°C.
y = -0.078x + 5.6161
R² = 0.9902
32.5°C.
y = -0.529x + 5.6939
R² = 1
37°C.
y = -0.7167x + 5.637
R² = 1
4°C.
Linear (21°C.)
21°C.
Linear (32.5°C.)
32.5°C.
Linear (37°C.)
37°C.
Linear (4°C.)

FIG. 10I


EFFECT OF TEMPERATURE ON MONOMER DISSOCIATION
HBc
26-35L
26-35
27-35
K off in hours
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
T°C.